CHAPTER 2. PREPARATION AND ANALYSIS OF DNA

CHAPTER 2. INTRODUCTION

Introduction

The ability to prepare and isolate pure DNA from a variety of sources is an important step in many molecular biology protocols. Indeed, the isolation of genomic, plasmid, or DNA fragments from restriction digests and polymerase chain reaction (PCR) products has become a common everyday practice in almost every laboratory. This chapter therefore begins with protocols for purification of genomic DNA from bacteria, plant cells, and mammalian cells (*UNITS* 2.1-2.4). These protocols consist of two parts: a technique to lyse the cells gently and solubilize the DNA, followed by one of several basic enzymatic or chemical methods to remove contaminating proteins, RNA, and other macromolecules. The basic approaches described here are generally applicable to a wide variety of starting materials. A brief collection of general protocols for further purifying and concentrating nucleic acids is also included.

The last decade has shown a dramatic departure from the use of traditional DNA purification methods outlined in *UNITS* 2.2-2.4, with a concomitant increase in the use of purpose-specific kits for the isolation and purification of DNA. For example, kits for purification of DNA using pre-made anion-exchange columns packaged with all necessary solutions to lyse the cells and solubilize the DNA are available from many molecular biology companies. A variety of kits based on binding of DNA to glass beads are also available. The uses of both types of kits are discussed in *UNIT 2.1B*.

The use of kits has two main advantages: it saves time and makes the process of DNA purification a relatively easy and straightforward process. The purification of DNA by anion-exchange chromatography (*UNIT 2.1B*) is readily becoming

the accepted standard for quick and efficient large-scale (more than 100 ug of DNA) production of DNA from bacteria, mammalian tissue, and plant tissue. In most cases, the cell lysis and solubilization of DNA is relatively unchanged compared to traditional methods, with anion-exchange chromatography columns having replaced labor and time-intensive techniques such as cesium chloride centrifugation for the isolation of relatively pure DNA. Purification kits are usually available in several sizes and configurations, allowing the researcher to have variability concerning the processing and purification of their DNA.

A variety of techniques exist for the isolation of small amounts of plasmid DNA from minipreps and for DNA fragments from restriction digests/PCR products from agarose gels (with removal of unincorporated nucleoside triphosphates, reaction products, and small oligonucleotides from PCR reactions). These are detailed in *UNITS* 2.1A, 2.1B, 2.6 & 2.7. Likewise, kits are available from several molecular biology companies, usually based on silica-gel technology, for each of these applications (*UNIT 2.1B*). As with large-scale DNA isolation and purification, these kits provide a quick and efficient means to recover purified DNA that can be used for subsequent cloning or other modifications.

Virtually all protocols in molecular biology require, at some point, fractionation of nucleic acids. Chromatographic techniques are appropriate for some applications and may be used for separation of plasmid from genomic DNA as well as separation of genomic DNA from debris in a cell lysate (*UNIT 2.1B*). Gel electrophoresis, however, has much greater resolution than alternative methods and is generally the fractionation method of choice. Gel electrophoretic separations can be either analytical or preparative, and can involve fragments with molecular weights ranging from less than 1000 Daltons to more than 10^8. A variety of electrophoretic systems have been developed to accommodate such a large range of applications.

In general, the use of electrophoresis to separate nucleic acids is simpler than its application to resolve proteins. Nucleic acids are uniformly

negatively charged and, for double-stranded DNA, reasonably free of complicating structural effects that affect mobility. A variety of important variables affect migration of nucleic acids on gels. These include the conformation of the nucleic acid, the pore size of the gel, the voltage gradient applied, and the salt concentration of the buffer. The most basic of these variables is the pore size of the gel, which dictates the size of the fragments that can be resolved. In practice, this means that larger-pore agarose gels are used to resolve fragments >500 to 1000 bp (*UNITS* 2.5A & 2.6) and smaller-pore acrylamide or sieving agarose gels (*UNIT 2.7*) are used for fragments <1000 bp. A protocol for resolution of very large pieces of DNA may also be resolved on agarose gels using pulsed-field gel electrophoresis (*UNIT 2.5B*). Finally, the powerful analytical technique of capillary electrophoresis of DNA (*UNIT 2.8*) may be used to assess the purity of synthetic oligonucleotides, analyze quantitative PCR results, and compare DNA fragment lengths from restriction fragment length polymorphism (RFLP) and variable number of tandem repeat (VNTR) analyses.

Frequently it is desirable to identify an individual fragment in a complex mixture that has been resolved by gel electrophoresis. This is accomplished by a technique termed Southern blotting, in which the fragments are transferred from the gel to a nylon or nitrocellulose membrane and the fragment of interest is identified by hybridization with a labeled nucleic acid probe. Section IV of this chapter gives a complete review of methods and materials required for immobilization of fractionated DNA (*UNIT 2.9*) and associated hybridization techniques (*UNIT 2.10*). These methods have greatly contributed to the mapping and identification of single and multicopy sequences in complex genomes, and facilitated the initial eukaryotic cloning experiments.

Other commonly encountered applications of gel electrophoresis include resolution of single-stranded RNA or DNA. Polyacrylamide gels containing high concentrations of urea as a denaturant provide a very powerful system for resolution of short (<500-nucleotide) fragments of single-stranded DNA or RNA. Such gels can resolve fragments differing by only a single

nucleotide in length, and are central to all protocols for DNA sequencing (see *UNIT 7.6*). Such gels are used for other applications requiring resolution of single-stranded fragments, particularly including the techniques for analyzing mRNA structure by S1 analysis (*UNIT 4.6*), ribonuclease protection (*UNIT 4.7*), or primer extension (*UNIT 4.8*). Denaturing polyacrylamide gels are also useful for preparative applications, such as small-scale purification of radioactive single-stranded probes and large-scale purification of synthetic oligonucleotides (*UNIT 2.12*).

Resolution of relatively large single-stranded fragments (>500 nucleotides) can be accomplished using denaturing agarose gels. This is of particular importance to the analysis of mRNA populations by northern blotting and hybridization. A protocol for use of agarose gels containing formaldehyde in resolution of single-stranded RNA is presented in *UNIT 4.9*. The use of denaturing alkaline agarose gels for purification of labeled single-stranded DNA probes is described in *UNIT 4.6*.

Gels and Electric Circuits

Gel electrophoresis units are almost always simple electric circuits and can be understood using two simple equations. Ohm's law, $V = IR$, states that the electric field, V (measured in volts), is proportional to current, I (measured in milliamps), times resistance, R (measured in ohms). When a given amount of voltage is applied to a simple circuit, a constant amount of current flows through all the elements and the decrease in the total applied voltage that occurs across any element is a direct consequence of its resistance. For a segment of a gel apparatus, resistance is inversely proportional to both the cross-sectional area and the ionic strength of the buffer. Usually the gel itself provides nearly all of the resistance in the circuit, and the voltage applied to the gel will be essentially the same as the total voltage applied to the circuit. For a given current, decreasing either the thickness of the gel (and any overlying buffer) or the ionic strength of the buffer will increase resistance and, consequently, increase the voltage gradient across the gel and the electrophoretic mobility of the sample.

A practical upper limit to the voltage is usually set by the ability of the gel apparatus to dissipate heat. A second useful equation, $P = I^2R$, states that the power produced by the system, P (measured in watts), is proportional to the resistance times the square of the current. The power produced is manifested as heat, and any gel apparatus can dissipate only a particular amount of power without increasing the temperature of the gel. Above this point small increases in voltage can cause significant and potentially disastrous increases in temperature of the gel. It is very important to know how much power a particular gel apparatus can easily dissipate and to carefully monitor the temperature of gels run above that level.

Two practical examples illustrate applications of the two equations. The first involves the fact that the resistance of acrylamide gels increases somewhat during a run as ions related to polymerization are electrophoresed out of the gel. If such a gel is run at constant current, the voltage will increase with time and significant increases in power can occur. If an acrylamide gel is being run at high voltage, the power supply should be set to deliver constant power. The second situation is the case where there is a limitation in number of power supplies, but not gel apparati. A direct application of the first equation shows that the fraction of total voltage applied to each of two gels hooked up in series (one after another) will be proportional to the fraction of total resistance the gel contributes to the circuit. Two identical gels will each get 50% of the total voltage and power indicated on the power supply.

Finally, it should be noted that some electrophoretic systems employ lethally high voltages, and almost all are potentially hazardous. It is very important to use an adequately shielded apparatus, an appropriately grounded and regulated power supply, and most importantly, common sense when carrying out electrophoresis experiments.

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From Current Protocols in Molecular Biology Online

APPENDIX 2 COMMONLY USED REAGENTS AND EQUIPMENT

BUFFERS AND STOCK SOLUTIONS

Introduction

This collection describes the preparation of buffers and reagents used in the manipulation of nucleic acids and proteins (see Table A.2.1). When preparing solutions, use deionized, distilled water and reagents of the highest grade available. Sterilization—by filtration through a 0.22-um filter or by autoclaving—is recommended for most applications. Recipes for the following can be found elsewhere in the manual: culture media (*UNIT 1.1*), antibiotics (Table 1.4.1), lactose analogs (Table 1.4.2), and enzyme buffers (*UNIT 3.4*).

CAUTION: *Handle strong acids and bases carefully.*

Acid precipitation solution

1 M HCl
0.1 M sodium pyrophosphate

Nucleic acids can also be precipitated with a 10% (w/v) solution of trichloroacetic acid (TCA); however, this recipe is cheaper, easier to prepare, and just as efficient.

Ammonium acetate, 10 M

Dissolve 385.4 g ammonium acetate in 150 ml H_2O
Add H_2O to 500 ml

BBS (BES-buffered solution), 2×

50 mM *N,N*-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid (BES; Calbiochem)
280 mM NaCl
1.5 mM Na_2HPO_4, pH 6.95
800 ml H_2O
Adjust pH to 6.95 with room temperature 1 N NaOH

H_2O to 1 liter

Filter sterilize through a 0.45-um nitrocellulose filter (Nalgene)

Store in aliquots at -20°C (can be frozen and thawed repeatedly)

The pH of this solution is critical (pH 6.95 to 6.98).

When a new batch of 2× BES buffer is prepared, its pH should be checked against a reference stock prepared (and tested) earlier.

$CaCl_2$, 1 M

147 g $CaCl_2 \cdot 2H_2O$
H_2O to 1 liter

Denhardt solution, 100×

10 g Ficoll 400
10 g polyvinylpyrrolidone
10 g bovine serum albumin (Pentax Fraction V; Miles Laboratories)
H_2O to 500 ml

Filter sterilize and store at -20°C in 25-ml aliquots

Dithiothreitol (DTT), 1 M

Dissolve 15.45 g DTT in 100 ml H_2O

Store at -20°C

EDTA (ethylenediamine tetraacetic acid), 0.5 M (pH 8.0)

Dissolve 186.1 g $Na_2EDTA \cdot 2H_2O$ in 700 ml H_2O
Adjust pH to 8.0 with 10 M NaOH (~50 ml)
Add H_2O to 1 liter

Ethidium bromide, 10 mg/ml

Dissolve 0.2 g ethidium bromide in 20 ml H_2O

Mix well and store at 4°C in dark

CAUTION: Ethidium bromide is a mutagen and must be handled carefully.

HBSS (Hanks balanced salt solution)

5.4 mM KCl
0.3 mM Na_2HPO_4
0.4 mM KH_2PO_4
4.2 mM $NaHCO_3$
1.3 mM $CaCl_2$

0.5 mM $MgCl_2$
0.6 mM $MgSO_4$
137 mM NaCl
5.6 mM D-glucose
0.02% phenol red (optional)
Add H_2O to l liter and adjust pH to 7.4

HBSS can be purchased from Biofluids or Whittaker.

HBSS may be made or purchased without $CaCl_2$ and $MgCl_2$. These are optional components that usually have no effect on an experiment. In some cases, however, their presence may be detrimental to a procedure. Consult the individual protocol to see if the presence or absence of these components is recommended in the materials list.

HCl, 1 M

Mix in the following order:
913.8 ml H_2O
86.2 ml concentrated HCl

HeBS (HEPES-buffered saline) solution, 2×

16.4 g NaCl
11.9 g HEPES acid
0.21 g Na_2HPO_4
800 ml H_2O
Titrate to pH 7.05 with 5 N NaOH
Add H_2O to 1 liter
Filter sterilize through a 0.45-um nitrocellulose filter
Test for transfection efficiency and store at -20°C in 50-ml aliquots

An exact pH is extremely important for efficient transfection. The optimal pH range is 7.05 to 7.12.

KCl, 1 M

74.6 g KCl
H_2O to 1 liter

$MgCl_2$, 1 M

20.3 g $MgCl_2 \cdot 6H_2O$
H_2O to 100 ml

$MgSO_4$, 1 M

24.6 g $MgSO_4 \cdot 7H_2O$
H_2O to 100 ml

MOPS buffer

0.2 M MOPS [3-(*N*-morpholino)-propanesulfonic acid], pH 7.0
0.5 M sodium acetate
0.01 M EDTA

Store in the dark and discard if it turns yellow

NaCl, 5 M

292 g NaCl
H_2O to 1 liter

NaOH, 10 M

Dissolve 400 g NaOH in 450 ml H_2O
Add H_2O to 1 liter

PBS (phosphate-buffered saline)

10× stock solution, 1 liter:
80 g NaCl
2 g KCl
11.5 g $Na_2HPO_4 \cdot 7H_2O$
2 g KH_2PO_4
Working solution, pH ~7.3:
137 mM NaCl
2.7 mM KCl
4.3 mM $Na_2HPO_4 \cdot 7H_2O$
1.4 mM KH_2PO_4

Potassium acetate buffer, 0.1 M

Solution A: 11.55 ml glacial acetic acid/liter (0.2 M).

Solution B: 19.6 g potassium acetate ($KC_2H_3O_2$)/liter (0.2 M).

Referring to Table A.2.2 for desired pH, mix the indicated volumes of solutions A and B, then dilute with H_2O to 100 ml.

This may be made as a 5- or 10-fold concentrate by scaling up the amount of potassium acetate in the same volume. Acetate buffers show concentration-dependent pH changes, so check concentrate pH by diluting an aliquot to the final concentration.

To prepare buffers with pH intermediate between the points listed in Table A.2.2, prepare closest higher pH, then titrate with solution A.

Potassium phosphate buffer, 0.1 M

Solution A: 27.2 g KH_2PO_4 per liter (0.2 M).

Solution B: 34.8 g K_2HPO_4 per liter (0.2 M).

Referring to Table A.2.3 for desired pH, mix the indicated volumes of solutions A and B, then dilute with H_2O to 200 ml.

This may be made as a 5- or 10-fold concentrate by scaling up the amount of potassium phosphate in the same volume. Phosphate buffers show concentration-dependent pH changes, so check concentrate pH by diluting an aliquot to the final concentration.

SDS electrophoresis buffer, 5×

15.1 g Tris base
72.0 g glycine
5.0 g SDS
H_2O to 1000 ml

Dilute to 1× or 2× for working solution, as appropriate

Store up to 1 month at 0° to 4°C

Do not adjust the pH of the stock solution, as the solution is pH 8.3 when diluted.

SED (standard enzyme diluent)

20 mM Tris·Cl, pH 7.5
500 ug/ml bovine serum albumin (Pentax Fraction V)

10 mM α-mercaptoethanol

Store at 4°C for up to 1 month

Sodium acetate, 3 M

Dissolve 408 g sodium acetate·$3H_2O$ in 800 ml H_2O
Add H_2O to 1 liter

Adjust pH to 4.8 or 5.2 (as desired) with 3 M acetic acid

Sodium acetate buffer, 0.1 M

Solution A: 11.55 ml glacial acetic acid/liter (0.2

M).
Solution B: 27.2 g sodium acetate ($NaC_2H_3O_2 \cdot 3H_2O$)/liter (0.2 M).

Referring to Table A.2.2 for desired pH, mix the indicated volumes of solutions A and B, then dilute with H_2O to 100 ml. (See Potassium acetate buffer recipe for further details.)

Sodium phosphate buffer, 0.1 M

Solution A: 27.6 g $NaH_2PO_4 \cdot H_2O$ per liter (0.2 M).
Solution B: 53.65 g $Na_2HPO_4 \cdot 7H_2O$ per liter (0.2 M).

Referring to Table A.2.3 for desired pH, mix the indicated volumes of solutions A and B, then dilute with H_2O to 200 ml. (See Potassium phosphate buffer recipe for further details.)

SSC (sodium chloride/sodium citrate), 20×

3 M NaCl (175 g/liter)
0.3 M Na_3citrate$\cdot 2H_2O$ (88 g/liter)
Adjust pH to 7.0 with 1 M HCl

STE buffer

10 mM Tris·Cl, pH 7.5
10 mM NaCl
1 mM EDTA, pH 8.0

TAE (Tris/acetate/EDTA) electrophoresis buffer

50× stock solution:
242 g Tris base
57.1 ml glacial acetic acid
37.2 g $Na_2EDTA \cdot 2H_2O$
H_2O to 1 liter
Working solution, pH ~8.5:
40 mM Tris·acetate
2 mM $Na_2EDTA \cdot 2H_2O$

TBE (Tris/borate/EDTA) electrophoresis buffer

10× stock solution, 1 liter:
108 g Tris base (890 mM)
55 g boric acid (890 mM)
40 ml 0.5 M EDTA, pH 8.0 (see recipe; 20 mM)

TE (Tris/EDTA) buffer

10 mM Tris·Cl, pH 7.4, 7.5, or 8.0 (or other pH;

see recipe)
1 mM EDTA, pH 8.0

TEA (triethanolamine) solution

50 mM triethanolamine, pH ~11.5
0.1% Triton X-100
0.15 M NaCl

Add Triton X-100 as a 10% stock sterilized by Millipore filtration and stored in the dark to prevent photooxidation (stock is stable 5 years at room temperature).

TEN (Tris/EDTA/NaCl) solution

40 mM Tris·Cl, pH 7.5 (*APPENDIX 2*)
1 mM EDTA, pH 8.0 (*APPENDIX 2*)
150 mM NaCl
Store up to 6 months at room temperature

TM buffer, 10×

100 mM Tris·Cl, pH 8.0
100 mM $MgCl_2$

Tris-buffered saline (TBS)

100 mM Tris·Cl, pH 7.5 (*APPENDIX 2*)
0.9% (w/v) NaCl (150 mM)
Store up to several months at 4°C

Tris·Cl [tris(hydroxymethyl)aminomethane], 1 M

Dissolve 121 g Tris base in 800 ml H_2O
Adjust to desired pH with concentrated HCl
Mix and add H_2O to 1 liter

Approximately 70 ml of HCl is needed to achieve a pH 7.4 solution, and approximately 42 ml for a solution that is pH 8.0.

NOTE: *The pH of Tris buffers changes significantly with temperature, decreasing approximately 0.028 pH units per 1°C. Tris-buffered solutions should be adjusted to the desired pH at the temperature at which they will be used. Because the pK_a of Tris is 8.08, Tris should not be used as a buffer below pH ~7.2 or above pH ~9.0.*

TTBS (Tween 20/TBS)

0.1% Tween 20 in Tris-buffered saline (TBS; see

recipe)
Store up to several months at 4°C

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BASIC PROTOCOL: PHENOL EXTRACTION AND ETHANOL PRECIPITATION OF DNA

This protocol describes the most commonly used method of purifying and concentrating DNA preparations. The DNA solution is first extracted with a phenol/chloroform/isoamyl alcohol mixture to remove protein contaminants, and then precipitated with 100% ethanol. The DNA is pelleted after the precipitation step, washed with 70% ethanol to remove salts and small organic molecules, and resuspended in buffer at a concentration suitable for further experimentation.

Materials

≤1 mg/ml DNA to be purified
25:24:1 (v/v/v) phenol/chloroform/isoamyl alcohol (made with buffered phenol; see Support Protocol 1)
3 M sodium acetate, pH 5.2 (*APPENDIX 2*)
100% ethanol, ice cold
70% ethanol, room temperature
TE buffer, pH 8.0 (*APPENDIX 2*)
Speedvac evaporator (Savant)

1. Add an equal volume of phenol/chloroform/isoamyl alcohol to the DNA solution to be purified in a 1.5-ml microcentrifuge tube.

DNA solutions containing ≤0.5 M monovalent cations can be used. Extracting volumes ≤100 ul is difficult; small volumes should be diluted to obtain a volume that is easy to work with.

High salt concentrations can cause the inversion of the aqueous and organic phases. If this happens, the organic phase can be identified by its yellow color.

2. Vortex vigorously for 10 sec and microcentrifuge 15 sec at maximum speed, room temperature.

Phases should be well separated. If DNA solution is viscous or contains a large amount of protein, it should be microcentrifuged longer (1 to 2 min).

3. Carefully remove the top (aqueous) phase containing the DNA using a 200-ul pipettor and

transfer to a new tube. If a white precipitate is present at the aqueous/organic interface, repeat steps 1 to 3.

If starting with a small amount of DNA (<1 ug), recovery can be improved by reextracting the organic phase with 100 ul TE buffer, pH 8.0. This aqueous phase can be pooled with that from the first extraction.

4. Add 1/10 vol of 3 M sodium acetate, pH 5.2, to the solution of DNA. Mix by vortexing briefly or by flicking the tube several times with a finger.

If the solution contained a high concentration of NaCl or sodium acetate (0.3 to 0.5 M) prior to the phenol extraction step, then no additional salt should be added. It is advisable to make appropriate dilutions to keep NaCl and sodium acetate concentrations below 0.5 M.

For high concentrations of DNA (>50 to 100 ug/ml), precipitation is essentially instantaneous at room temperature. If ethanol precipitation is not desirable, residual organic solvents can be removed by ether extraction (see Support Protocol 3). In this case, no salt should be added.

To prevent carryover of residual phenol, the aqueous phase can be reextracted with 24:1 (v/v) chloroform/isoamyl alcohol. However, this should not be necessary if the final pellet is washed well with 70% ethanol, or if an additional ethanol precipitation step is included.

5. Add 2 to 2.5 vol (calculated *after* salt addition) of ice-cold 100% ethanol. Mix by vortexing and place in crushed dry ice for 5 min or longer.

This precipitation step can also be done in a -70°C freezer for 15 min or longer, or in a -20°C freezer for at least 30 min. A slurry of dry ice and ethanol may also be used, but tube labels are less often lost when crushed dry ice is used.

6. Microcentrifuge 5 min at maximum speed and remove the supernatant.

For large pellets the supernatant can simply be poured off. For small pellets (<1 ug), aspirate off the ethanol supernatant with a pipetting device such as a Pasteur pipet or mechanical pipettor. This is best accomplished by drawing off liquid from the side of the tube opposite that against which the DNA precipitate was pelleted. Start at the top and move downward as the liquid level drops.

7. Add 1 ml room-temperature 70% ethanol. Invert the tube several times and microcentrifuge as in step 6.

If the DNA molecules being precipitated are very small (<200 bases), use 95% ethanol at this step.

8. Remove the supernatant as in step 6. Dry the pellet in a desiccator under vacuum or in a Speedvac evaporator.

The DNA pellet will not stick well to the walls of the tube after the 70% ethanol wash and care must be taken to avoid aspirating the pellet out of the tube.

9. Dissolve the dry pellet in an appropriate volume of water if it is going to be used for further enzymatic manipulations requiring specific buffers. Dissolve in TE buffer, pH 8.0, if it is going to be stored indefinitely.

DNA pellets will not dissolve well in high-salt buffers. To facilitate resuspension, the DNA concentration of the final solution should be kept at <1 mg/ml.

If DNA is resuspended in a volume of TE buffer or water to yield a DNA concentration of <1 mg/ml, small quantities (<25 ug) of precipitated plasmids or restriction fragments should dissolve quickly upon gentle vortexing or flicking of the tube. However, larger quantities of DNA may require vortexing and brief heating (5 min at 65°C) to resuspend. High-molecular-weight genomic DNA may require one to several days to dissolve and should be shaken gently (not vortexed) to avoid shearing, particularly if it is to be used for cosmid cloning or other applications requiring high-molecular-weight DNA. Gentle shaking on a rotating platform or a rocking apparatus is recommended.

ALTERNATE PROTOCOL 1: PRECIPITATION OF DNA USING ISOPROPANOL

Equal volumes of isopropanol and DNA solution are used in precipitation. Note that the isopropanol volume is half that of the given volume of ethanol in precipitations. This allows precipitation from a large starting volume (e.g., 0.7 ml) in a single microcentrifuge tube. Isopropanol is less volatile than ethanol and takes longer to remove by evaporation. Some salts are less soluble in isopropanol (compared to ethanol) and will be precipitated along with nucleic acids. Extra washings may be necessary to eliminate these contaminating salts.

SUPPORT PROTOCOL 1: PREPARATION OF BUFFERED PHENOL AND PHENOL/CHLOROFORM/ISOAMYL ALCOHOL

For some purposes, fresh liquefied phenol (88% phenol) can be used without further purification. However, for purification of DNA prior to cloning and other sensitive applications, phenol must be redistilled before use, because oxidation products of phenol can damage and introduce breaks into nucleic acid chains. Redistilled phenol for use in nucleic acid purification is commercially available, but must be buffered before use. Appropriately buffered phenol is also commercially available, but is somewhat more expensive and should not be stored for long periods of time (e.g., >6 months).

CAUTION: Phenol can cause severe burns to skin and damage clothing. Gloves, safety glasses, and a lab coat should be worn whenever working with phenol, and all manipulations should be carried out in a fume hood. A glass receptacle should be available exclusively for disposing of used phenol and chloroform.

Materials

8-hydroxyquinoline
Liquefied phenol
50 mM Tris base (unadjusted pH ~10.5)
50 mM Tris·Cl, pH 8.0 (*APPENDIX 2*)
Chloroform
Isoamyl alcohol

1. Add 0.5 g of 8-hydroxyquinoline to a 2-liter glass beaker containing a stir bar.

2. Gently pour in 500 ml liquefied phenol or melted crystals of redistilled phenol (melted in a water bath at 65°C).

The phenol will turn yellow due to the 8-hydroxyquinoline, which is added as an antioxidant.

3. Add 500 ml of 50 mM Tris base.

4. Cover the beaker with aluminum foil. Stir 10 min at low speed with magnetic stirrer at room temperature.

5. Let phases separate at room temperature.

Gently decant the top (aqueous) phase into a suitable waste receptacle. Remove what cannot be decanted with a 25-ml glass pipet and a suction bulb.

6. Add 500 ml of 50 mM Tris·Cl, pH 8.0. Repeat steps 4 to 6 so that two successive equilibrations with Tris·Cl are performed, ending with removal of the second Tris·Cl phase.

The pH of the phenol phase can be checked with indicator paper and should be 8.0. If it is not, the Tris·Cl equilibration should be repeated until this pH is obtained.

7. Add 250 ml of 50 mM Tris·Cl, pH 8.0, or TE buffer, pH 8.0, and store at 4°C in brown glass bottles or clear glass bottles wrapped in aluminum foil.

Phenol prepared with 8-hydroxyquinoline as an antioxidant can be stored ≤2 months at 4°C.

8. For use in DNA purification procedure (see Basic Protocol), mix 25 vol phenol (bottom yellow phase of stored solution) with 24 vol chloroform and 1 vol isoamyl alcohol. Store up to 2 months at 4°C wrapped in foil or in a dark glass bottle.

SUPPORT PROTOCOL 2: CONCENTRATION OF DNA USING BUTANOL

It is generally inconvenient to handle large volumes or dilute solutions of DNA. Water molecules (but not DNA or solute molecules) can be removed from aqueous solutions by extraction with *sec*-butanol (2-butanol). This procedure is useful for reducing volumes or concentrating dilute solutions before proceeding with the Basic Protocol.

Additional Materials (also see Basic Protocol)

sec-Butanol
25:24:1 (v/v/v) phenol/chloroform/isoamyl alcohol (made with buffered phenol; see Support Protocol 1)
Polypropylene tube

1. Add an equal volume of *sec*-butanol to the sample and mix well by vortexing or by gentle inversion (if the DNA is of high molecular weight). Perform extraction in a polypropylene tube, as butanol will damage polystyrene.

2. Centrifuge 5 min at 1200 × *g* (2500 rpm), room temperature, or in a microcentrifuge for 10 sec at maximum speed.

3. Remove and discard the upper (*sec*-butanol) phase.

4. Repeat steps 1 to 3 until the desired volume of aqueous solution is obtained.

5. Extract the lower, aqueous phase with 25:24:1 phenol/chloroform/isoamyl alcohol and ethanol precipitate (see Basic Protocol, steps 1 to 9) or remove *sec*-butanol by two ether extractions (see Support Protocol 3).

Addition of too much sec-butanol can result in complete loss of the water phase into the sec-butanol layer. If this happens, add 1/2 vol water back to the sec-butanol, mix well, and spin. The DNA can be recovered in this new aqueous phase and can be further concentrated with smaller amounts of sec-butanol.

The salt concentration will increase in direct proportion to the volume decrease. The DNA can

be precipitated with ethanol to readjust the buffer conditions.

SUPPORT PROTOCOL 3: REMOVAL OF RESIDUAL PHENOL, CHLOROFORM, OR BUTANOL BY ETHER EXTRACTION

DNA solutions that have been purified by extraction with phenol and chloroform (see Basic Protocol) or concentrated with *sec*-butanol (see Support Protocol 2) can often be used without ethanol precipitation for enzymatic manipulations or in gel electrophoresis experiments if the organic solvents are removed by extraction with ether. Traces of ether are subsequently removed by evaporation. This procedure is useful only if the solute concentrations in the starting solution are compatible with what is needed in later steps. It is quite useful in purifying high-molecular-weight DNA, as mechanical shearing of large nucleic acid molecules can occur during precipitation with ethanol.

CAUTION: Ether is highly flammable and its vapors can cause drowsiness. All manipulations with ether should be carried out in a well-ventilated fume hood.

Materials

Diethyl ether
TE buffer, pH 8.0 (*APPENDIX 2*)
Polypropylene tube

1. Mix diethyl ether with an equal volume of water or TE buffer, pH 8.0, in a polypropylene tube. Vortex vigorously for 10 sec and let the phases separate.

Ether is the top phase.

2. Add an equal volume of hydrated ether to the DNA sample. Mix well by vortexing or by gentle inversion (if the DNA is of high molecular weight).

3. Microcentrifuge 5 sec at maximum speed or let the phases separate by setting the tube upright in a test tube rack.

4. Remove and discard the top (ether) layer. Repeat steps 2 and 3.

5. Remove ether by leaving the sample open under a fume hood for 15 min (small volumes, <100 ul), or under vacuum for 15 min (larger

volumes).

The DNA solution will be free of organic solvents and will have salt concentrations that are roughly three-fourths of those that were in the aqueous solution before phenol extraction (solute concentrations are lowered in the two phenol/chloroform/isoamyl alcohol extractions steps).